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Embryonic Stem Cells

TEC-1 CHARACTERISATION OF PORCINE EMBRYONIC CELLS
FROM DAY 11 EMBRYONIC DISCS CULTURED IN SERUM-FREE MEDIUM

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Day-11 porcine embryonic discs express cell phenotype markers typical of undifferentiated murine embryonic stem cells (ESCs): they are positive for the stage specific marker TEC-1/SSEA-1 (a potential marker of totipotency), but are negative for cytokeratin 8/18. However, culture of such discs in serum-containing medium induces partial differentiation whereby expression of TEC-1 disappears; whereas, cytokeratin expression becomes evident (Wianny et al., 1995, Soc. Franç. Étud. Fert. 34^e Réun., Montpellier, Abst. C2). This differentiation may be induced by the growth factors and retinoids contained in serum. Hence, a serum-free medium was developed to re-examine TEC-1 expression patterns in primary cultures of porcine embryonic disc cells derived from day-11 blastocysts.

Embryonic discs were microsurgically dissected free of trophectoderm and endoderm and cultured on a fibronectin substrate (1 µg/cm²) in DMEM/F12 medium supplemented with 10 µg/ml insulin, 5.5 µg/ml transferrin, 6.7 ng/ml selenium, 0.1 mM β-mercaptoethanol and either 10 or 100 ng/ml human leukaemia inhibitory factor (LIF): addition of 100 ng/ml LIF to this medium permitted 24/44 (54.5%) of attached embryonic discs to continue to expand after the third day in culture; whereas, 0/26 (0%) of discs cultured in only 10 ng/ml LIF survived, as all detached from the substrate at this time. Colonies cultured in 100 ng/ml LIF were stained for TEC-1 from the 3-5th day of culture. Compared to typical colonies cultured in the presence of serum, remarkably different TEC-1 expression patterns and cellular morphologies were observed in serum-free medium. Three major cell phenotypes could be identified: a) cells possessing a high nucleo-cytoplasmic ratio that appeared to be directly derived from the embryonic disc that were variably stained for TEC-1. Such cells appeared to be differentiating into two other cell types: b) epithelial-like polygonal cells staining both positively and negatively for TEC-1, so as to produce a mosaic-type staining pattern, c) cells of low nucleo-cytoplasmic ratio that formed rapidly expanding populations that were negative for TEC-1. TEC-1 positive cell populations accounted for 32.3 ± 31% (mean ± sd) of the total area of the colonies (n=16). Individual addition of other growth factors (10 ng/ml basic fibroblastic growth factor (n=8 colonies), 100 ng/ml epidermal growth factor (n=18) or 100 ng/ml insulin-like growth factor-II (n=10)) together with LIF (100 ng/ml) did not increase the percentage area represented by the TEC-1 positive cell populations (24.9 ± 33, 31.3 ± 30, and 29.4 ± 28%, respectively, P > 0.05).

These results indicate: i) high concentrations of LIF are essential to maintain colonies in serum-free medium, ii) removal of serum (or specific serum components) permits continued expression of TEC-1 which, if analogous to murine ESCs, suggests a maintenance of totipotency in embryonic disc-derived cultured cells. Further examination of the growth factor requirements that can specifically expand these TEC-1 positive populations is required.

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